# Analysis of Therapeutic Peptides by HPLC

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## Abstract

Insulin is a hormone, which is present in all vertebrates. In humans its biosynthesis takes place in the pancreas. Insulin and its antagonist, glucagon are responsible for the control of blood sugar. Insulin lowers and glucagon raises the blood sugar level. Insulin consists of two strains A and B, which are connected by a disulfide bridge.

Oxytocin influences uterus contractions. It is used in both human and veterinary medicine to start contractions.Together with renine and aldosterone, angiotensin I and II play a major role in regulating blood pressure, circulation and diuresis.

Figure 1 shows the HPLC separation of angiotensin I, angiotensin II, oxytocin and insulin (chain B from bovine) using gradient analysis on a reversed phase column and UV detection. The autosampler temperature was set to 4 ºC to avoid decomposition of the samples.

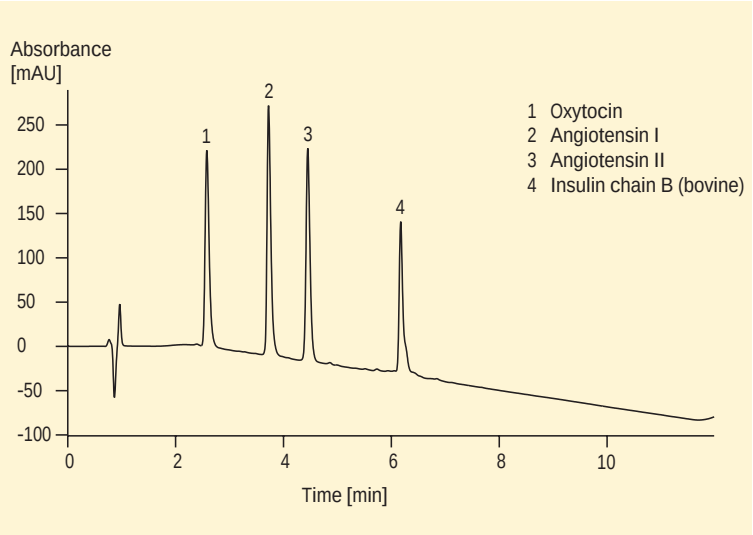


Figure 1
Analysis of angiotensin I, angiotensin II, oxytocin and insulin (chain B from bovine)

## Conditions

**Column**
4.6 x 75 mm Zorbax SB-C18 3.5 µm
**Mobile phase**
A = 0.1 % TFA in water
B = acetonitrile
**Flow rate** 1.0 ml/min
**Gradient** 20 % B to 50 % B in 10 min.
**Column wash** 50 % B to 20 % B in 2 min
**UV detector**
variable wavelength detector
210 nm, standard cell
**Column compartment temperature**
25 °C
**Stop time** 12 min
**Post time** 5 min
**Injection volume** 5 µl

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The performance of the HPLC method is shown in the table below.

| Compound | LOD for S/N=2 (mg/l)* | Precision of RT (RSD of 10 runs) (250 mg/l)* | Precision of Area (RSD of 10 runs) (250 mg/l)* |
|---|---|---|---|
| Oxytocine | 2.5 | 0.11 | 0.38 |
| Angiotensin I | 2.5 | 0.07 | 0.17 |
| Angiotensin II | 2.5 | 0.08 | 0.14 |
| Insulin chain B (bovine) | 2.5 | 0.05 | 0.17 |

* Injection volume: 5 µl

The HPLC method presented here shows an easy but reliable and precise analysis of the therapeutic peptides angiotensin I, angiotensin II, insulin (chain B, bovine) and oxytocin. The values for LOD, precision of RT and precision of area show the good performance of the analysis.

## Equipment

**Agilent 1100 Series**

- Quaternary pump (includes vacuum degasser)
- Thermostatted autosampler
- Thermostatted column compartment
- Variable wavelength detector, standard flow cell, 10-mm path length, 13-µl cell volume

*Alternative:*

- Vacuum degasser
- Binary pump
- Diode-array detector, standard flow cell 10-mm path length, 13-µl cell volume
- Agilent ChemStation + 3D software

**Columns**

- Zorbax SB-C18, 3.5 µm, 4.6 x 75 mm (Agilent part number 866953-902)
- Recommended: Guard cartridges Zorbax SB-C18, 5 µm, 4.6 x 12.5 mm (Agilent part number 820950-920, 4/pk)

*Note:*
Since the method was specifically developed on the Agilent 1100 Series system you might not be able to reproduce this analysis on an older system or even on a new system with lower performance. To avoid sample decomposition it is necessary to use a cooled autosampler, for example, the Agilent 1100 Series thermostatted autosampler.

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